CLAIMS

What is claimed:

1. A method of producing a templated extracellular matrix, comprising the steps of:

providing a nanostructured artificial template; and

contacting the nanostructured artificial template with a population of cells activated for producing a templated extracellular matrix.

2. The method of claim 1 where the artificial template comprises a biocompatible texture surface.

3. The method of claim 1 wherein the artificial template comprises one of aligned polymer etched silicon, textured polymers, etched semi-conductor material, and glass.

4. The method of claim 1 wherein the templated extracellular matrix is used for generating one of corneal stroma and other structured connective tissue such as a ligament, a tendon, a fascia and annulus fibrosis.

5. A method of producing a templated extracellular matrix, comprising the steps of:

controlling a flow of a polymer solution into a device having a substrate, the device generating a shear flow to induce alignment of polymer structures;

controlling a plurality of parameters during polymerization;

generating a first layer of nanostructured artificial template;

contacting the first layer of nanostructured artificial template with a first population of cells; and

maintaining the nanostructured artificial template and the first population of cells in a culture to produce a templated extracellular matrix.

6. The method of claim 5, wherein the polymer is a biopolymer such as collagen.

7. The method of claim 6, wherein the method further comprises the steps of:

mixing a solution of collagen with phosphate buffered saline solution;

adjusting the pH of the solution to 7.4 ± 0.2;

applying the solution at a controlled rate onto a substrate which generates a shearing flow;

causing preferential orientation of the self-assembling collagen fibrils; and

generating successive layers, each layer representing a portion of the component.

8. The method of claim 7, wherein the layers have a uniform, controllable thickness ranging from sub-micron to 100 microns.

9. The method of claim 6, wherein the collagen is type I and/or type V collagen.

10. The method of claim 5, wherein the principle orientation of the aligned fibrils in a single layer alternates in each successive layer.

11. The method of claim 7, wherein the angle between the principle orientation of each layer is approximately in the range of 0 to 180 degrees.

12. The method of claim 5, wherein the solution properties, including temperature, concentration and surfactant composition are controlled.

13. The method of claim 5, wherein the shear flow is generated by spinning the substrate at a controlled rate in a range of approximately 50 to 50,000 Hz.

14. The method of claim 5, wherein the shear flow is generated by drawing the substrate out of the collagen solution.

15. The method of claim 5, wherein the atmosphere is controlled to a specified temperature and relative humidity.

16. The method of claim 5, wherein the solution conditions are modulated to control the polymerization kinetics and morphology.

17. The method of claim 5, wherein the use of shear flow aligns polymerizing polymer chains in a layer such that polymers are predominantly aligned parallel to each other.

18. The method of claim 5, further comprising angular rotation of the substrate providing shear flow and confinement to orient the polymerized polymers.

19. The method of claim 18, wherein an input flow rate, solution viscosity and substrate rotational velocity combine to produce a shear rate between 1 s^{-1} and 500,000 s^{-1}.

20. The method of claim 18, wherein an input flow rate, solution viscosity and substrate rotational velocity combine to produce a shear rate preferably between the range 10 s^{-1} and 10,000 s^{-1}.

21. The method of claim 5, wherein an additional layer comprising collagen type IV and cell adhesion proteins such as, laminin, fibronectin and/or any integrin receptor is deposited between or onto aligned polymer layers.

22. The method of claim 5, wherein a construct of a plurality of aligned layers is used as a replacement or repair of the human corneal stroma.

23. The method of claim 7, wherein the alignment of the polymers in a plane of second and subsequent layers is predominantly parallel with the alignment of the polymers in a plane of the first layer.

24. The method of claim 7, wherein the alignment of the polymers in a plane of a layer in a second and subsequent layers is predominantly orthogonal with the alignment of the polymers in the plane of the first layer.

25. The method of claim 7, wherein the alignment of the polymers in a plane of a layer in the second and subsequent layers does not have a defined angular relationship to the alignment of the polymers in a plane the first layer.

26. The method of claim 5, wherein the monomer is included in an aqueous solution.

27. The method of claim 26, wherein the monomer is collagen.

28. The method of claim 26, wherein the monomer is extracted or recombinant collagen.

29. The method of claim 27, wherein the collagen is Type I as the polymerizing medium.

30. The method of claim 27, wherein the collagen is Type I and Type V to assist in creation of heterotypic fibrils.

31. The method of claim 5, wherein the polymer solution is injected at a constant rate.

32. The method of claim 5, wherein the polymer solution is injected with a flow rate between 0.05-1000 ml/min.

33. The method of claim 5, wherein the material is preferably injected with a flow rate between of 0.1-100.0 ml/min.

34. The method of claim 5, further comprising a post-processing step including spinning off any effluent material from the substrate.

35. The method of claim 5, further comprising the substrate and a substrate holder being modified to minimize waste of polymerization solution.

36. The method of claim 5, wherein the solution is preferably composed of 8:1:1 ratio of collagen type I (3 mg/ml) to 10x PBS to 0.1M NaOH with pH adjusted to 7.4.

37. The method of claim 5, wherein the viscosity of the solution is between 1 mPa.s and 100 Pa.s.

38. The method of claim 5, where the viscosity solution is preferably between 5 mPa.s and 1 Pa.s.

39. The method of claim 5, wherein the substrate comprises one of a flat surface or curved surface.

40. The method of claim 39, wherein the flat surface is optically smooth.

41. The method of claim 39, wherein preferably the flat surface has a surface roughness of approximately less than 10 microns.

42. The method of claim 39, wherein the substrate is a borosilicate glass disk.

43. The method of claim 5, wherein a surface of the substrate is treated to control adhesion of the polymer and wetting of the solution.

44. The method of claim 5, wherein a surface of the substrate is ultrasonicated in 10% micro90 (Brand) cleaner for a time duration.

45. The method of claim 5, wherein a surface of the substrate is plasma cleaned.

46. The method of claim 5, wherein a surface of the substrate is homogeneous.

47. The method of claim 5, wherein the substrate has a surface treatment that is heterogeneous.

48. The method of claim 5, wherein the substrate has a surface treatment that is patterned.

49. The method of claim 5, wherein a substrate is patterned to constrain the flow.

50. The method of claim 5, wherein a surface of the substrate and atmospheric conditions are modulated to control self-assembly.

51. The method of claim 5, wherein additives are injected with the polymer solution to control the polymerization process and final morphology of the layer.

52. The method of claim 51, wherein the additives are proteoglycans.

53. The method of claim 51, wherein the additives are at least one of chondroitin sulfate, dermatan sulfate and keratan sulfate proteoglycans.

54. The method of claim 51, wherein the proteoglycans are one of at least or a combination of decorin, lumican, biglycan, keratocan or syndican.

55. The method of claim 51, wherein the percent (by weight) of added proteoglycans is between 0.25 and 50.0.

56. The method of claim 51, wherein the percent by weight of added proteoglycans is between 0.5 and 10.

57. A method of producing a templated extracellular matrix, comprising the steps of:

providing a nanostructured artificial template;

contacting the nanostructured artificial template with a first population of cells; and

maintaining the nanostructured artificial template and the first population of cells in culture to produce a templated extracellular matrix having a first surface and a second surface.

58. The method of claim 57, further comprising the step of stacking a plurality of templated extracellular matrix layers oriented at any arbitrary angle with respect to each other to form a multilaminar templated extracellular matrix having a first surface and a second surface.

59. The method of claim 58 wherein the multilaminar templated extracellular matrix layer is a biomimetic corneal stroma.

60. The method of claim 58, further comprising the steps of:

contacting the first surface of the multilaminar templated extracellular matrix with a second population of cells; and

maintaining the multilaminar templated extracellular matrix and the second population of cells in culture to produce a multilaminar templated extracellular matrix having layer of the second population of cells on the first surface.

61. The method of claim 60, further comprising the steps of:

contacting the second surface of the multilaminar templated extracellular matrix with a third population of cells; and

maintaining the multilaminar templated extracellular matrix and the third population of cells in culture to produce a multilaminar templated extracellular matrix having layer of the third population of cells on the second surface.

62. The method of claim 57 wherein the cells are mammalian cells.

63. The method of claim 57 wherein the cells are mammalian fibroblasts.

64. The method of claim 60 wherein the cells are mammalian cells.

65. The method of claim 60 wherein the cells are corneal epithelial cells.

66. The method of claim 63 wherein the mammalian fibroblasts are activated by treatment with ascorbic acid, pharmacologically acceptable organic and inorganic ascorbate salts and ascorbate esters.

67. The method of claim 66 wherein the activated fibroblasts are made quiescent by the remote of ascorbate.

68. The method of claim 61 wherein the cells are mammalian cells.

69. The method of claim 61 wherein the cells are corneal endothelial cells.

70. The method of claim 57 wherein the nanostructured artificial template is unstressed.

71. The method of claim 57 wherein the nanostructured artificial template is subjected to tensile stress.

72. The method of claim 60 wherein the multilaminar templated extracellular matrix is unstressed.

73. The method of claim 60 wherein the multilaminar templated extracellular matrix is subjected to tensile stress.

74. The method of claim 61 wherein the templated extracellular matrix is unstressed.

75. The method of claim 61 wherein the templated extracellular matrix is subjected to tensile stress.

76. The method of claim 57 wherein the nanostructured artificial template comprises collagen.

77. The method of claim 76, wherein the nanostructured artificial template further comprises proteoglycans.

78. The method of claim 76, wherein the nanostructured artificial template further comprises at least one of chondroitin sulfate, dermatan sulfate and keratan sulfate proteoglycans.

79. The method of claim 76, wherein the proteoglycans are one of at least or a combination of decorin, lumican, biglycan, keratocan or syndican.

80. The method of claim 76, wherein the percent (by weight) of proteoglycans is between 0.25 and 50.0.

81. A biomimetic corneal stroma produced by the steps of:

providing a nanostructured artificial template;

contacting the nanostructured artificial template with a first population of eukaryotic cells;

maintaining the nanostructured artificial template and the first population of cell in culture to produce a templated extracellular matrix;

repeating the steps of providing, contacting and maintaining to produce additional templated extracellular matrices; and

stacking a plurality of templated extracellular matrices oriented at any arbitrary angle with respect to each other.

82. The biomimetic corneal stroma of claim 81 wherein the eukaryotic cells are mammalian fibroblasts.

83. The biomimetic corneal stroma of claim 81 wherein the eukaryotic cells are human keratocytes.

84. The biomimetic corneal stroma of claim 81 further comprising the step of treating the eukaryotic cells with an ascorbate compound selecting from the

group consisting of ascorbate acid, pharmaceutically acceptable organic and inorganic salts of ascorbate, organic and inorganic esters of ascorbate and mixtures thereof.

85. The biomimetic corneal stroma of claim 84 further comprising the step of removing the ascorbate compound.

86. A biomimetic cornea produced by the steps of:

providing a nanostructured artificial template;

contacting the nanostructured artificial template with a first population of eukaryotic cell;

maintaining the nanostructured artificial template with the eukaryotic cells to form a template extracellular matrix;

repeating the steps of providing, contacting and maintaining to form additional templated extracellular matrices;

stacking a plurality of templated extracellular matrices oriented at any arbitrary angle with respect to one another to form a multilaminar templated extracellular matrix;

contacting a first surface of the multilaminar templated extracellular matrix with a second population of cells; and

maintaining the multilaminar templated extracellular matrix in culture to form a biomimetic cornea.

87. A method of making a multilaminar nanostructured template comprising:

introducing a monomer solution from a first inlet, between a polymer accepting surface and a polymer rejecting surface to first outlet to produce an aligned polymer layer;

increasing the spacing between the polymer accepting surface and the polymer rejecting surface;

introducing the monomer solution into a second inlet and recovering the monomer solution from a second outlet wherein the flow from the second inlet to the second outlet is substantially orthogonal to the flow from the first inlet to the first outlet; and

producing an aligned polymer layer in which the polymer molecules are substantially orthogonal to the polymer molecules of the previous layer.

88. The method of claim 87 wherein the polymer is collagen.

89. The method of claim 87 wherein the polymer rejecting surface is cooled.

90. The method of claim 87 wherein the polymer accepting surface is heated.